NOTES ON THE BACTERIOLOGICAL EXAMINATION OF WATER FROM THE SYDNEY SUPPLY. No. I.

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(Plates X. and XI.)

(Introductory.)

When, some time ago, through the generous and ever-ready assistance of the Hon. William Macleay, who not only defrayed the cost of the necessary working-apparatus, but also encouraged me in my pursuits in every possible way, and to whom I take this opportunity of tendering my best thanks, I was fortunate enough to commence to do some bacteriological work in the laboratory at the Linnean Hall, Sydney, I fancied that, among other interesting subjects, the Sydney water, as used for drinking and other purposes, might be worth a biological or more especially a bacteriological examination.

It is well-known that bacteria or Schizomycetes (fission-or cleft-fungi) occur in most natural waters, and also, that these very micro-organisms are possessed of very marked physiological properties, which manifest themselves in different ways, as processes of oxidation and hydratation, of fermentation and putrefaction, according to the chemical changes which they bring about in various substances. Last but not least, a pretty fair number of bacteria claim a more than usual interest, inasmuch as they are indubitably proved to be intimately connected with the production of certain, so-called infectious diseases in man, animals, and, to some extent in the vegetable world, while in other such diseases the existence of the real contagium in the form of a micro-organism, has not yet been clearly demonstrated, but can only be inferred.

Representatives of all these groups of bacteria may be contained in or transported by drinking-water, which, on account of its being a liquid, constitutes an admirable vehicle for them.

Some importance, therefore, ought always to be attached to the testing of potable waters for bacteria, more especially since plain and convenient, and at the same time, satisfactory methods of research are now at our disposal. In cases and at times of epidemics especially, for instance of typhoid fever, such inquiries are undoubtedly extremely useful, as they may supply us with facts, otherwise scarcely or not attainable.

The water which formed the subject of the present examination was pipe-water from the Sydney supply, and was derived, in nearly all cases, from the tap in the laboratory at the Linnean Hall; one sample only was obtained from a tap in Mr. Macleay's house.

The remarks made in this paper on the condition of that water as regards the bacteria found in it, do not by any means pretend to be exhaustive; they are, in fact, but the results of some observations briefly relating to the quantity, and some characteristic features of the micro-organisms hitherto obtained, and they will in time, I hope, be followed by data of a more comprehensive nature.

Methods of Examination.

In examining the water under consideration I employed Koch's method, with which I had ample opportunity of making myself acquainted in Germany during the year 1885. The principle which underlies this method, and in which it so materially differs from all other methods relative to the same subject—I shall do well to state that here in a few words—consists in the application of a solid and at the same time transparent, nutrient soil for the cultivation of vegetable micro-organisms in their pure state, *i. e.* not mixed with foreign elements. In this respect, the most universal cultivating medium, as used by the school of Koch, is a 5%-10% meat-broth-peptone-gelatine, or shortly nutrient gelatine, which is still solid at a temperature of 25° C. (77° F.) This nutritive gelatine—I need scarcely say here that in conducting pure cultures a thorough sterility of all substances and apparatus used, is a *conditio sine*

qua non—in a liquid state, and having a temperature at any rate not higher than blood-heat, is mixed with whatever it is desired to test for micro-organisms. In our case, a definite quantity of water is well distributed in a certain quantity of nutrient gelatine in a test-tube, and the still liquid mixture is then, with the adoption of due precautions, of course, transferred to and spread on sterilised glass-plates, which, after sufficient solidification of the layer of gelatine has taken place, are placed in a convenient form of damp chamber, and therein subjected to temperatures not exceeding 25° C. (77° F.), for a certain period. The great advantages of this mode of carrying on bateriological examinations are, in the main :—

(1.) The *modus operandi* is extremely simple and free from the concomitant complications of other methods regarding bacteriology.

(2.) The whole of the germs in the sample of water, or whatever it may be operated upon in the described manner, are deposited all at once on the culture-plates ; they become, each of them, fixed to a separate spot in or on the solidifying gelatine. Here those capable of development in the gelatine—most of the Schizomycetes are—go to form groups or colonies which are not all alike, but according to the specifically different germs from which they originate, differ from one another, generally even to the naked eye. In these colonies or vegetations the bacterial species are distinguishable from one another, just in the same way as "a number of birds in their flights, or socially living ants in their wanderings."

(3.) From these colonies inoculations on or into various nutritive media for the purpose of obtaining pure cultivations, can be easily and successfully carried out.

On the other hand, Koch's method of gelatine-plate-cultivation for the bacteriological investigation of water, is not altogether devoid of some sources of error, which seem to be mainly these :—

(1.) There are a few groups of bacteria which refuse to grow at all in nutrient gelatine, or, at least, within the limits of temperature for solid gelatine. Parasitic species especially, *e.g. Bacillus tuberculosis* (Koch), will not be found to multiply under this treatment, nor will the forms which are grouped together under the

name of Spirobacteria (Spirillum, Spirochaete). Then again, exclusively anaerobic bacteria, such as the bacillus of butyric acid fermentation, and the bacillus of malignant œdema, will not, at least under ordinary circumstances, develop in nutrient gelatine.

These groups, however, it must be admitted, form only a small part of the whole class of bacteria; yet it would be very important, at any rate, to have in the gelatine-plate process a means for their detection. By far the greater majority of bacteria, as already mentioned, grow readily in or on the common nutrient gelatine, Koch's comma-bacillus of cholera asiatica, and the bacillus of typhoid fever (Eberth) can, if present in the water under examination, scarcely escape notice. (*)

(2.) It is not always justifiable to regard the number of bacterial colonies met with on the plates of gelatine as corresponding exactly to as many individual germs in the sample of water under consideration. Bacteria, as is well known, have a tendency to form various kinds of aggregations, or to combine in groups of growth which are not always so easily separable into their individual components. Therefore, as von Malapert-Neufville proposes (†) the best way to say is :—

One cubic-centimeter of the water used
in the experiment a, yielded A bacterial colonies.
" " b, " B " "
and so on.

A few other objections to Koch's method of water-test are but of a slight and immaterial character; they can be satisfactorily met by paying the strictest care and attention to the prescribed course and manner of manipulation.

Before examining the water which, as already stated above, was derived from a tap in the Linnean Hall, and once only from one in

(*) Conf. also Robert Freiherr von Malapert-Neufville, "Bacteriolog. Untersuchung d. wichtigsten Quellen d. städtischen Wasserleitung Wiesbadens und einer Anzahl Mineral-Quellen" . . . . . Zeitschrift f. Analytische Chemie von Fresenius, Jahrg. 25, Heft. 1, Wiesbaden 1886, pp. 39-88.

(†) Loc. cit.

Mr. Macleay's house, it was always allowed to run to waste for some time, after which about 50 cubic centimeters of it were collected in sterilised, small so-called Erlenmeyer's or parting flasks of about 130 ccm. capacity. Immediately after that procedure plate-cultivations were made, for which purpose mostly 1 ccm., besides that sometimes $\frac{1}{2}$ ccm. and $\frac{1}{4}$ ccm., of the samples of water were added to the gelatine kept in test-tubes.

As cultivation-plates I employ glass plates, about 11 cm. long, and 8 cm. broad, and a more satisfactory and convenient shape is arrived at by giving them the form of an octagon (Plates X, XI., fig. 1, 2, 3, 4). The damp chambers used by me consist of two flat glass-dishes of strong, white glass, with perpendicular walls. One of them measures 14-15 cm. inner diameter, and 6-6·5 cm. inner height or depth; it is destined for holding one or more of the culture-plates. The other, 15·5 cm. inner diam. and 3 cm. inner height or depth, is inverted and serves as cover for the former.

The incubator for low temperatures up to 25° C. (77° F.), in the laboratory, is made after a suitable design for such incubators; and, when necessary, the required temperature was kept up by means of a small kerosene-flame.

For plate-cultivations I employed a 10 % nutrient gelatine; for test-tube cultivations a 5%-6% gelatine (1). The microscopical examinations are made with a microscope by R. Winkel, Göttingen, Germany, having $\frac{1}{14}$ homogeneous immersion-objective, and condensing apparatus.

A direct microscopical examination of the samples under consideration was usually not made, for, however important such an examination might appear theoretically, yet its application is attended with such a variety of disadvantages that, after all,

(1) It was not possible for me to get in Sydney that variety of French gelatine which is recommended for the cultivation of Schizomycetes. So I took, from want of something better, a pretty good French gelatine (black and gold label; Coignet Père & Fils & Cie, Paris). As to the dry peptone, an essential although only small ingredient (1%) in nutrient gelatine, I was not able to obtain it here at all. I, therefore, had recourse to preparing as much as 1 oz myself; I employed it in a not quite dry state. A supply of it and of other material (especially gelatine) is now on its way to me from Germany.

it cannot furnish precise results, and, therefore, cannot be of comparatively great value for the bacteriological analysis of water.

Number of Colonies of Bacteria.

In the following table I give a brief account of the number of bacterial colonies which made their appearance on the cultivation-plates, after a period of from two to three days' incubation, at a temperature of about 20° C. (68° F.) They are always calculated, in the now customary and conventional way for one cubic centimeter (1) of the samples under treatment. These figures have been arrived at by a series of single experiments, made on 14 different samples, within the space of about two months. This is indicated by the date when the sample had been taken. Moreover, I noted the temperature of the water operated upon, and besides the amount of bacterial colonies in general, I thought it well not to omit to state in a special column the number of those colonies which caused liquefaction of the gelatine. It is especially bacteria of this kind which induce fermentative and putrefactive processes in organic substances, although there are, on the other hand, important pathogenic species of bacteria, *e.g.*, the bacillus of typhoid fever (Eberth), which do not liquefy the nutrient gelatine in the least.

Date.	Temper. of Water.	Number of Colonies in 1 ccm.	Liquefying Colonies in 1 ccm.
(1) July 14 (?)	(?)	167	166 = 100 p.c.
(2) ,, 19	(?)	140	132 = $94\frac{2}{7}$ p.c.
(3) ,, 29	51° F. = $10\frac{5}{9}$°C.	69	24 = $34\frac{3}{4}$ p.c.
(4) Aug. 4	51 F. = $10\frac{5}{9}$ C.	2000	180 = 9 p.c.
(5) ,, 8	52 F. = $11\frac{1}{9}$ C.	1960	42 = $2\frac{1}{7}$ p.c.
(6) ,, 13	53 F. = $11\frac{2}{3}$ C.	500	174 = $34\frac{4}{5}$ p.c.
(7) ,, 18	$51\frac{1}{4}$ F. = $10\frac{2}{3}$ C.	520	334 = $64\frac{3}{13}$ p.c.
(8) ,, 23	51 F. = $10\frac{5}{9}$ C.	120	24 = 20 p.c.
(9) ,, 28	54 F. = $12\frac{2}{9}$ C.	35	6 = $17\frac{1}{7}$ p.c.
(10) Sept. 2	55 F. = $12\frac{7}{9}$ C.	23	0 = 0 p.c.
(11) ,, 7	60 F. = $15\frac{5}{9}$ C.	160	70 = $43\frac{3}{4}$ p.c.
(12) ,, 10	57 F. = $13\frac{8}{9}$ C.	38	4 = $10\frac{1}{2}$ p.c.
(13) ,, 16	59 F. = 15 C.	107	48 = $44\frac{7}{8}$ p.c.
(14) ,, 21	57 F. = $13\frac{8}{9}$ C.	51	3 = 6 p.c.

(1) 1 cubic centim. (ccm.) = ·060242 cub. inch.

These figures yield, out of the 14 single cases, an average number of 421 colonies in 1 ccm., and among these, 86 liquefying ones, equal to $20\frac{3}{7}$ p.c.

From the above table it will be seen that we have, as regards the bacterial colonies, numbers before us which fluctuate within rather considerable limits. The maximum of colonies enumerated was 2000 on August 4th, the minimum 23 on September 2nd. On Aug. 8th, the likewise enormous number of 1960 was obtained; twice (Aug. 13th and 18th), 500 and 520 respectively. In five cases the numbers oscillate between 100 and 200, whereas in only five cases out of the whole were there less than 100 colonies.

The amount of bacterial life in a given sample of water is, under otherwise quite the same circumstances, greatly dependent on the amount of organic matter suspended in it. In other words: the more bacteria in the water, the greater the amount of organic matter in it. Now it is worth notice that the numbers 2000, 1960, 500, 520, as stated above, were obtained on days which succeeded a period of rather heavy rain. This rainfall carried or washed into the supply (dams) a certain quantity of organic detritus along with the accompanying micro-organisms, and, after a time, the consequences of this addition to the pipe-water made themselves evident by an enormous increase in the quantity of bacterial colonies. The water under examination then improved again vastly, as is well seen in all the remaining cases (see above).

From this also it follows that a general judgment of any water, with reference to its contained bacteria, cannot be arrived at by one single test, made on one or another day. Such an isolated experiment will give us nothing but a rough idea of the condition of the water for a limited time, and is not to be generalised. Even the above 14 individual cultivations have to be multiplied, in order to get more correct and reliable average numbers which would admit of even a general verdict.

As regards the relation between the quantity of bacteria present in a given water, and its quality from a sanitary point of view, as

a potable water, Professor Koch says (1):—"A large number of micro-organisms indicates that the water has received admixtures in a state of decomposition and loaded with micro-organisms, impure tributaries, etc., which might contribute in addition to the many harmless bacteria, also pathogenic forms, that is, infectious matter.

. . Experience thus far has shown that in good waters the number of germs capable of development varies between 10 and 150. As soon as the number considerably exceeds this limit, the water must be suspected of receiving contributions from polluted sources. If the number reaches or exceeds 1000 I should not permit its use as drinking water, at least not in time of a cholera epidemic. The number 1000 is chosen by me as arbitrarily as has been the case in selecting the limiting values in chemical analysis, and I allow each one to change it according to his convictions."

After these statements of Koch the particular tap-water of the Sydney supply—yielding an average number of 421 bacterial colonies in 1 ccm., for a period of little more than two months (see above)—cannot be declared as good.

Description of the bacteria.

In what follows I shall briefly describe the forms of bacteria hitherto obtained from the above-mentioned pipe-water. This description relates :—

(1). To the appearances of the bacteria under high powers of the microscope. The specimens are taken from colonies on the glass-plates, and examined both living and after having been stained with Loeffler's alkaline methyleneblue-solution.

(2). To the morphological features exhibited by the different species

(a). in their colonies on plates of gelatine, both with regard to their naked-eye appearances, and to those visible and demonstrable by the application of low powers of the microscope (70-122 diam. ; transmitted light ; [narrow diaphragm.])

(1) The original text not being at hand, I quote a translation communicated in the "American Monthly Microscopical Journal." Vol. VII., Washington, April 1886, No. 4, p. 64.

(b). in their mode of growth in solid 5-6 % nutritive gelatine enclosed in test-tubes, or in other words, in the deportment of their pure cultivation in this nourishing soil and under these conditions.

(c). in their pure cultivation on an oblique surface of peptonised agar-agar broth or nutrient agar-agar (1) in test-tubes.

Five specifically different forms of bacteria as yet have been with certainty obtained from the water in question ; they are described provisionally as Bacterium (Bacillus or whatever it may be) A, B, C, &c., adopting the plan of Malapert-Neufville. (2)

Of a few other bacteria the colonies of which from time to time appeared on the gelatine-plates, it is more than doubtful that they were contained in the samples of water employed. Firstly, they were met with in mostly one colony each, and only very seldom ; secondly, they made their appearance after the plates, for the purpose of first examination, had already been in contact with the air of the room ; lastly, they were found only at the surface of the gelatine. These colonies showed themselves to be very interesting, and I hope to return to them at the earliest opportunity.

Bacillus A.

Microscopical Characters. Short rods of from ·0015-·0018 mm. (3) in length and about ·0008 in breadth ; singly or in twos ; extremities rounded ; protoplasmic contents not thoroughly homogeneous, inasmuch as the ends of the rods stain with aniline-dye better than

(1) Agar-Agar, or Japanese isinglass, of good quality, is to be had in packets of about ¼ lb. at Chinese shops, George-street, Sydney. According to a label referring to a sample of such vegetable isinglass in the Technological Museum of Sydney, it is called " Kanten " " prepared by exposing the jelly obtained from *Gelidium corneum* (Lamarck) to the intense frost of a winter's night. It congeals and hardens and may then be kept for a great length of time." So far as I am informed there are more than this one species of seaweeds used for preparing Japanese isinglass or agar-agar. A nutrient agar-agar of 1 % withstands more than blood-temperature without becoming liquid, and is, on account of this property and from the fact that it is not to be liquefied by any bacterial growth, much used in bacteriological laboratories.

(2) Loc. cit.

(3) 1 mm. = ·03937 inch ; ·001 mm. = ·0003937 inch.

their central parts. With very active spontaneous movements which exhibit themselves as a tremulous hurrying across the field of the microscope, and are especially extremely vivid in the immediate neighbourhood of air-bubbles in a drop of water under the cover-glass (ærobic bacterial form.)

On gelatine-plates. It forms at the surface of the gelatine greyish, turbid-looking colonies of circular circumference (Pls. X, XI, figs. 1, 3, 4, a), which exhibit, when examined with low magnifying powers, granular contents. The colonies enlarge very rapidly, liquefying at the same time and at the same rate the gelatine, effecting in the latter, at first, funnel-shaped, then with the advancing growth of the micro-organism, watch-glass-like excavations now filled with liquid matter. In the interior of the layer of gelatine the colonies multiply much more slowly than do the superficial ones.

When quite young—of from ·01-·12 mm. diam.—the colonies present, in optical section, more or less perfect circle-figures, with smooth outlines, as indicated by a bright, black, uninterrupted line, and showing a greyish colour. Contents of the vegetations homogeneous, slightly granular. In the larger ones signs of a commencing liquefaction of gelatine are visible.

In *nutrient gelatine* in a *test-tube* this bacillus displays a vigorous propagation which results in the production, in a proportionately short time, and along the track of the inoculating platinum wire, of an inverted conical bag of liquefied gelatine. The growth rapidly spreads itself in the gelatine, forming at the bottom of the conical-shaped or forefinger-like excavation of the latter a granular, rather dense deposit, whilst in the superincumbent liquid, which offers a turbid greyish appearance, small granules and particles are distributed. In course of time, the whole contents of the test-tube become one liquid mass.

On a sloping surface of *nutrient agar-agar* in a test-tube this bacillus readily grows laterally from the streak of the inoculation, and ultimately represents a greyish-white, shining, gelatinous,

elongated, superficial layer, the edges of which are smooth and well defined, and thinner, and, therefore, more translucent than the other parts.

The culture of the above bacillus in or on the nutritive media here mentioned, did not cause any offensive smell.

With one or two exceptions, its colonies were always met with on the cultivation-plates sometimes in proportionately large, sometimes also in proportionately small numbers. It is the most common among the liquefying bacteria from the water.

Bacillus B.

Microscopical Characters. Short rods, ·002 mm. long and about ·0007 mm. thick. Occur singly or in twos; motile; extremities rounded off.

On gelatine-plates. At the surface of the gelatine the micro-organism grows in gelatinous, glistening, compact, but easily separable patches, (Pl. XI, fig. 3, 4 b,) which, in reflected light, and viewed from above, have a bluish-grey, in transmitted light (especially if condensed), and viewed from the side, a beautifully bluish-opalescent colour. Contours or edges quite irregularly shaped (Pl. XI. fig. 3, 4. b,). In the centre of these masses, as a rule, one finds a small, somewhat elevated part, forming as it were, a sort of nucleus, from which the spreading of the vegetation takes place. Under a low magnifying power the contents of these colonies look finely granular, and are translucent with a light grey tint.

In the interior of the layer of the gelatine this bacillus is met with in characteristic lenticular, or Cyclas- Anodonta- and Unio-like colonies, which are very often placed edgewise or obliquely in the mass of gelatine. (Pl. X, fig. 1, b; pl. XI, fig. 3, 4, b). They are of a nearly white colour, and rather viscid consistency. Under low magnifying power, and if not too old, these interior colonies are of a greyish colour (transmitted light), having their contents finely granular and their contours smooth. In their optical section they sometimes strikingly resemble the long contour of lemons.

When quite young—I examined them ·01-·08 mm. in diameter—the colonies are, in optical section, circular, translucent, with a light-grey colour, and possessed of perfectly smooth, well defined outlines. Contents homogeneous, slightly granulate.

In *nutrient gelatine* in a *test-tube* this bacillus grows pretty slowly to a whitish solid thread of equal dimensions throughout all its length. The contents of this thread are not homogeneous inasmuch as it appears to be made up, notably at its edges, of great numbers of larger or smaller beads. At the surface of the gelatine the growth is more marked, extending centrifugally beyond the point of inoculation, and forming a shining, irregularly indented film or pellicle of a bluish-grey colour.

This micro-organism causes no liquefaction whatever of the gelatine, neither in test-tubes, nor on plates.

On an *oblique surface* of *nutrient agar-agar* it grows readily, and when exposed in an incubator at blood-temperature it multiplies considerably, within less than two days, to indistinctly greyish-white, jelly-like, superficial layers which suddenly cease to increase in size, and do not extend all over the free surface of the nutritive soil. On microscopical examination endogenous spore-formation was found to exist. This bacterium usually made its appearance on the cultivation plates, supplying, on the average, the largest contribution to the whole of the bacteria cultivated.

At first sight of the colonies and test-tube cultivations of this bacillus, I thought of the possibility of its being perhaps the bacillus of typhoid fever. The microscopical appearances, are however, against such a possibility. I have not yet finished cultivating it on potatoes at blood-temperature, nor have I hitherto made with it any inoculation experiments on animals. In addition to that it would be of paramount importance to have as standards of comparison, for this and other similar forms which might be detected in Sydney water or elsewhere, pure cultivations of the *Bacillus typhosus*. Such a pure culture, also of other pathogenic Schizomycetes, I expect daily from Professor Flügge, Director of the Hygienic Institution at Göttingen University, Germany.

Bacillus C.

Microscopical Characters. Delicate, slender rods, of from ·0015 —·0035 mm. in length, and about ·0004 mm. in width; with somewhat acutely rounded ends; occur usually in threads or filaments, made up of a great number of individual rods.

On *gelatine-plates.* Growing superficially it forms, at first, very thin, irregularly shaped, opalescent films, which, under a low magnifying power, show a mosaic-like arrangement of their contents. Later on, with the moderately quickly advancing growth of the colonies, liquefaction of the gelatine sets in, and at the bottom of the watch-glass-like excavation in the latter, now a liquid mass, there is seen a net-work of ochre-yellow, rather thick and short strings which, taken as a whole are longitudinal or circular in shape (Pl. X, fig. 2, c). These colonies spread themselves peripherally more and more, as more or less elongated threads, which are combined in more or less wide and elongated bundles, these being themselves in communication with one another in the most various ways.

In the interior of the gelatine the colonies have, from the very beginning, a yellowish colour.

When in quite a young stage of development (of from ·02-·2 mm. diam.) the colonies of this bacillus seldom represent, as a whole, a circular shape (optical section), but they are mostly irregularly circumscribed, with their contents slightly emarginate and partly provided with offshoots, often of the most curious and fantastic kind, in so far as they resemble root-fibres, legs of mites and insects, or the like. Contents of these colonies granular, translucent with a yellowish tint.

In *nutrient gelatine* in a *test-tube* this form grows in the shape of an inverted, elongated cone which, if looked at in transmitted light, offers a beautiful aspect inasmuch as a central axis representing the course of the inoculating platinum-wire, appears to be beset, all round, with an almost invisible, extremely fine and delicate, cotton-wool-like mass, of a cloudy appearance. The growth here proceeds but slowly. At the surface of the gelatine the

micro-organism vegetates more quickly, liquefying the gelatine from above downwards, and forming at the bottom of the liquefied mass a dense ochre-yellow deposit.

On an *inclined surface* of *nutrient gelatine* in test-tubes it multiplies very readily, spreading laterally from the streak of the inoculation in a thin greyish-white film over the surface of the gelatine. On examination with a pocket-lens the edges of the growth are found to be lined with minute fringe-like processes, and, here and there, more or less elongated acuminate offshoots are seen which consist of an aggregation of minute, undulatory fibres, and are arranged, on each side, parallel to one another, running obliquely from below upwards, to the right and to the left respectively. The gelatine soon liquefies, first in a longitudinal, middle channel, carrying down with it to the bottom of the glass tube, the bacterial vegetation of these spots, and depositing it there as an orange-yellow dense flocky and rather tenacious mass. The liquefaction proceeds laterally till, after some time and at ordinary temperature, the test-tube is filled with one liquid mass. Besides the superficial growth, as observed in the gelatine-tube, of this bacillus, there exists, as long as the gelatine is solid, some inner vegetation, that is, from the gelatine-surface delicate, cloud-like, filamentous masses take their way into the solid gelatine in a parallel arrangement and in nearly a horizontal direction.

On a *sloping surface* of *nutrient agar-agar* this bacterium forms an ochre-yellow superficial layer with glistening even surface, and a narrow, thin, transparent, undulating border.

This species appeared now and then on the plates, but never copiously, fourteen colonies at one time being the largest number found (Pl. XI, fig. 2.)

Bacillus D.

Microscopical Characters. Cylindrical, straight or sometimes slightly curved rods, of from ·004-·009 mm. in length, and about ·0017 mm. in width ; occur singly, in twos, or in chains or filaments; extremities rounded off; with slow, seemingly pendulum-like, or slowly gliding, spontaneous movements ; contents of the rods homogeneous.

On *gelatine-plates.* This bacillus came under notice only a few times, and in few colonies. There is in the gelatine a watch-glass-like excavation, with perfectly circular circumference, and filled with turbid, liquefied gelatine, in which the colony (Pl. XI, fig. 4, d) is seen to consist of a central part of peculiar flocky, or sponge-like contents, and surrounding it a zone in which there are visible only small particles or granules, amidst the greyish, turbid gelatine-liquid. The spreading of the colonies, or what is the same, the liquefaction of gelatine takes place at a very rapid rate.

If, starting from such colonies, a fresh gelatine-plate is made, one finds very soon colonies of from ·05-·3 mm. in diameter. The superficial ones differ from the interior ones in that they are larger and already exhibit liquefaction of the gelatine, consisting of minute funnel-shaped openings in the latter. All the colonies, notably the deeper ones, are echinate in their appearance, in so far as from a central, on the whole circular mass (optical section) of more or less grey colour (transmitted light), there issue in different directions, more or less elongated, spine-or rod-like processes which represent a rather dense zone or girdle. A little below the surface of the gelatine the colonies sometimes give off small tuft-like off-shoots towards the surface of the gelatine. The quite superficial colonies are light-grey translucent.

In *nutrient-gelatine* in a *test-tube* the bacillus forms, at first liquefying the gelatine, a growth of the shape of an inverted elongated cone, that rapidly advances. At last there is a dense and thick deposit at the bottom of a columnar mass, consisting of turbid, liquid gelatine. I did not see the liquefaction go down entirely to the bottom of the test-tube, so that here part of the solid gelatine remained unaltered.

On an *oblique surface* of *agar-agar* it forms a quickly spreading compact, greyish-white, superficial layer, with its surface somewhat wrinkled, also here and there showing thin and pretty high folds, which extend more or less horizontally from the edges towards the middle of the growth. The marginal parts of the latter are curved and undulatory; the contours themselves are pretty smooth.

Bacillus E.

Microscopical Characters. About ·006 mm. long and ·0018 mm. broad cylindrical rods; occur in filaments, consisting of a great many individual rods, rounded at their extremities; no spontaneous movement.

On *gelatine-plates.* This form was only now and then met with in a few colonies. At the beginning they consist of but a few short threads crossing one another in various directions. They multiply pretty quickly, and after about three days present greyish, cloudy masses, having a central, darker, rounded part, where the gelatine is liquefied in a watch-glass-like manner, and from which delicate and multifariously ramified, and more or less elongated threads or filaments radiate, spreading themselves at a good distance over the surface of the gelatine. Besides that, I once saw a colony propagating in a similar manner at the bottom of the gelatine on the glass-plate. This micro-organism, therefore, is ærobic as well as anærobic.

In *nutrient gelatine* in a *test-tube* it grows, like Bacillus C, in the shape of an inverted cone, which, however, in this case approaches somewhat that of a cylinder. We have here a remarkably beautiful growth of extremely delicate, cloudy, and wool-fibre-like appearance; it is scarcely visible in reflected light, and reminds one vividly of a test-tube pure-cultivation of the bacillus of mice-septicaemia (Koch). It multiplies, in the interior of the gelatine, far more readily than does Bacillus C (see above). At the surface, where liquefaction of the gelatine begins, the micro-organism offers at first pretty much the same aspect as in its cultivation on plates; later on, with the advancing liquefaction of the gelatine, occurring from above downwards, it represents here a greyish-white film, covered by a liquid mass.

On a *sloping surface* of *nutrient agar-agar* it develops a luxuriant superficial vegetation of a grey-white colour. In the middle it is denser and more compact, being here folded up and provided with small prominences. At the edges it is thinner and has a lint-like appearance, being composed here of densely packed,

handsomely ramified threads or fibres which stretch over the whole available surface of the agar-agar on either side. Besides this superficial growth, there is also some growth in the interior of the agar-agar, inasmuch as short, cloudy masses penetrate from the surface into the substance of the solid agar-agar.

In concluding this first part of my Notes on Water from the Sydney Supply from a bacteriological point of view, I wish to state once more that they relate exclusively to the pipe-water of a single locality, of a locality where its quality might, of course, be altogether different from that at other places in or about the city. The number of bacteria in a given sample of water bears, under otherwise the same circumstances, as has been already mentioned above, a direct relation to the amount of organic matter in it, and this organic matter will or may not be equally distributed throughout the whole supply. Therefore it certainly would be erroneous to apply what could have been stated about the condition of the water of that locality to the whole supply in general. Further, the above statements as to the quantity of bacterial colonies in the sample under consideration are the results of cultivation experiments made during a comparatively cool and dry season. How the results will turn out during the hot summer or in wet periods in winter, cannot yet be exactly anticipated. Of special importance, of course, it would be in these bacteriological examinations by means of the gelatine-plate-process, always to have a watchful eye on whether the bacillus of typhoid fever, this social calamity, might with absolute certainty be found in the Sydney water, or not.

EXPLANATION OF PLATES.

Plate X.

Fig. 1.—Bacterial colonies growing in a gelatine plate-cultivation, out of 1 ccm. of pipe-water (July 19, 1886), after two days' incubation at about 65° F. (18⅓° C.) Natural size. (The layer of gelatine is represented here as in the following figures by a brownish tint).

a. Liquefying colonies of Bacillus A. (p. 915).

b. Non-liquefying colonies of Bacillus B. (p. 917).

Fig. 2.—Liquefying colonies (c) of Bacillus C. (p. 919), after several days. Natural size. The other colonies which were at the same time found on the plate are omitted (July 29th, 1886).

Plate XI.

Fig. 3.—Bacterial colonies from ¼ ccm. of water (Aug. 17th, 1886), after several days' incubation. Natural size.

a. Colonies of Bacillus A. (p. 915).

b. Interior
b,. Superficial } Colonies, non-liquefying, of Bacillus B. (p. 917).

Fig. 4.—Colonies from ½ ccm. of water (Aug. 23rd, 1886), after some days. Natural size.

a. Bacillus A.

b. b,. Bacillus B.

c. Bacillus C.

d. Liquefying colonies of Bacillus D.